STUDIES ON THE DIFFERENTIATION FROM THE CALLUS OF CICHORIEAE SPECIES

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Differentiation of the callus derived from the leaf segment of *Youngia denticulata* was reported previously (YAZAWA and TAKASHIMA 1989). This time the authors attempt to make the callus of other species in Cichorieae group and to analyse the variation of karyotype of various cells from the tissue culture primordia. SUNDERLAND (1973) reported the cells of the callus are genetically unstable. We have not obtained sufficient results in the chromosome study, so will preliminary report the observation of callus primordium.

Materials and Methods

Sonchus oleraceus used in this study for the material of callus formation is biennial plant, flowering in spring and passing from autumn to winter in the form of rosette leaf (Fig. 1). The somatic chromosome number is 32 ($2n=32$). This material was collected from its natural habitat.

Lactuca indica is collected from a habitat in Chiba prefecture and is used for the material of tissue culture.

The leaflets were cut off with a blade and sterilized by dipping into solution of sodium hypochlorite or ethanole.

The leaflets of the materials were cultured in a test tube in an incubator maintained at a temperature of 22°C and illuminated 14 hours by fluorescent tubes.

The primordia were maintained in the Murashige and Skoog's basic medium (containing 3% sugar and 0.2% Gellan gum (Gelrite)).

The medium K is Kinetin 5 ppm and IBA 10 ppm added in basic medium. Medium D is 2, 4-D 5 ppm and IBA 10 ppm added in basic medium. Medium O is only basic medium without any phytohormone added.

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Results

Sonchus oleraceus

The leaflet segments cut off from the leaf of *Sonchus oleraceus* (Fig. 1) were placed on each K-, D- and O-medium in May, 1989 and the formation of callus observed (Fig. 2). The callus developed well on K-medium. It seems K-medium is considerably suitable for development of callus of this species. Fig. 3 shows the callus developed at 70 days after the initial placement on the medium. The callus at the right in this photograph, transplanted from K-medium to O-medium developed root-like tissue. The portion of the callus in bright tone is white colored callus and made up of rather many cells in the mitotic figure.

Figures 6 through 10 show the various differentiation of calli. The arrow ⇨ in the figure indicates white callus and arrow ➡ is red-brown one. The letter Y shows yellow callus, G the green callus, L light green callus.

In Fig. 6 the primordia surrounded the white callus. In the lower side of Fig. 7 are several primordia which seem to be the root-like tissue. White calli gathered (Fig. 8), or green colored calli (Fig. 9) were assembled in clusters. Fig. 10 shows the callus tissues produced a light-green root with many white root hairs. Fig. 11 is a callus clump developed from the leaflet, from which the inner morphology of white callus in the center shows in Figs. 12–20.

Fig. 12. Cut down the white dome-like tissue and slightly pressed. In this figure can be seen the thick walled tracheid tissue surrounded spherically. In Fig. 14 on the left and Fig. 17 recognized epidermal tissue begin to appear. Inside of the callus shown in Fig. 14, tracheid-like cells or their cluster start to exist.

Lactuca indica

The calli formed from the section of leaflet of *Lactuca indica* in 1988 (Fig. 21) stopped developing owing to the secretion of a lot of latex. It then turned brown in color and finally died. A young matured leaf was used for the material this time. Segments of leaflet were placed on K-medium in the test-tube. After 18 days the calli developed (Fig. 22). Pretty masses of white and green calli formed on surface of the underside of the leaflet segment (Figs. 23, 24). Figs. 25, 26 showed the development of organ-differentiation from the callus on 106 days after. Green leaf and light brownish bud-like structures were recognized and only just differentiate from the root. Figs. 27–29 showed the after 10 days calli.

Figs. 30–36 were micrographs of the calli after 30 days from

degeneration of *Lactuca indica*. Arrows in the figures indicate the same as stated above.

In comparison with the case of *Sonchus oleraceus*, the dome-like bodies were numerous, and epidermal cells were definite (Fig. 33). We considered that the red-brownish calli were abundant for they were affected by the pigment generally including the somatic tissue of *Lactuca indica*.

The inner part of white or greenish primordia in Figs. 30-36 were various cell masses and mitotic figures of them were observed. Huge type cells were recognized (Figs. 39, 40) which contained yellow brown granules, owing to the cells becoming aged. So far as the observation of mitotic figures are concerned, they are almost normal and the number of chromosomes are $2n=18$ only.

Summary

From the leaflet segment of *Sonchus oleraceus* cultured on K medium, the callus formed smoothly. Calli developed well on K medium, and they differentiate into various forms and colors. The authors observed them through a stereo-microscope, and some of the photographs are included the text.

The calli consisted of light-green or white colored primordia mass, and developed root-like shoot structures. Epidermal tissue developed in white dome-like callus, in which tracheid cells or tissue developed. After a while somewhat long roots sprouted from it.

The callus of *Lactuca indica* were apt to become brown-colored from the effect of the secretion of latex from the cut surface of the leaflet. We obtained the callus of this species for the first time using for the material a young leaf segment on K medium.

Thereafter the callus grew well on K medium and granular primordia came out successively. White, light green, reddish purple cell clump were there, among them green dome-shape calli were conspicuous. These green dome-shaped calli consisted of many mitosis cells and the morphology of the cells were diverse. The leaflet or buds primordia developed and grew root-like shoot structures later on.

Literature

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Explanation of figures

Fig. 1. *Sonchus oleraceus* in experimental field.

Figs. 2–5. Callus induction and differentiation from leaf-explant of *Sonchus oleraceus*. ×1. The small letter at the left corner in the figure indicates the medium used. The basic medium is Murashige-Skoog's media, Gellan gum (0.2%) was used instead of agar. The medium K is Kinetin 5 ppm and IBA 10 ppm added in the basic medium. Medium D is 2,4-D 5 ppm and IBA 10 ppm added in basic medium. Medium O is only basic medium without any phytohormone added. 2. Difference of callus formation on various media. Left, medium K and right, medium D. 3. Left, on medium K; right, root formed on medium O after transplanted from medium K. 4, 5. Callus differentiated variously and shooting out many roots.

Figs. 6–10. Differentiation of calli of *Sonchus oleraceus*. The color of calli are varied in white, greenish and light brown and also differentiation takes place already. 6, 7. White callus are seen brightly in the figure; other parts were yellowish and green. The dark black part is red-brown callus. White process or root-like structure were seen. ×5.5. 8. White callus. ×25. 9. Green callus consists of a clump of cells. ×25. 10. From the callus greenish root-like structures emerged with many root hairs. ×25.

Fig. 11. Callus formation from the leaflet. White dome-like primodium is in the center, surrounded by yellow or brown callus cells. ×5.

Figs. 12–20. Microphotographs of white callus shown in center of Fig. 11. 12. Pack the portion of white callus with tracheid-like structure cells closely together. ×120. 13. Callus cells. 14. Epidermal cells on the left, and callus cells. ×40. 15, 16, 18–20. Various tracheid-like cells in inner part of callus. 15, 16, 20; ×250. 17; ×100. 18; ×500. 19; ×80.

Fig. 21. *Lactuca indica* in experimental field.

Figs. 22–29. Callus induction and leaf formation from leaf-explant of *Lactuca indica* on medium K. 22–24. Callus induction from leaf-explant. Callus 18 days after incubation. ×1. 25, 26. Differentiation from callus 95 and 115 days after incubation. 27–29. Callus cells from leaf explant 10 days after the incubation. ×240.

Fig. 30. Various calli of *Lactuca indica*. White colored and light green colored. ×40.

Fig. 31. Yellow callus developed on leaflet segment, and red brown callus seen in peripheral zone. ×12.

Fig. 32. Callus clumps in light green color with partially red-brown. ×4.

Fig. 33. Green callus with epidermal tissue.

Fig. 34. Light green or green colored callus and upper right in the photo is transparent primordia. ×12.

Fig. 35. White and brown colored calli; ➡ arrow indicates the red brown part. ×12.

Fig. 36. Yellow greenish callus; ➡ arrow indicates brown callus.

Figs. 37–42. Mitotic cells in calli of *Lactuca indica*. 37. Elongated cells often seen. ×370. 38. Cells in prophase. 39. Yellow brown granules in huge cell. 40. Same as Fig. 39 above. 41. Cells in prophase and anaphase show normal mitosis. 38–41. ×750. 42. Chromosomes of callus cell ($2n=18$). ×1500.

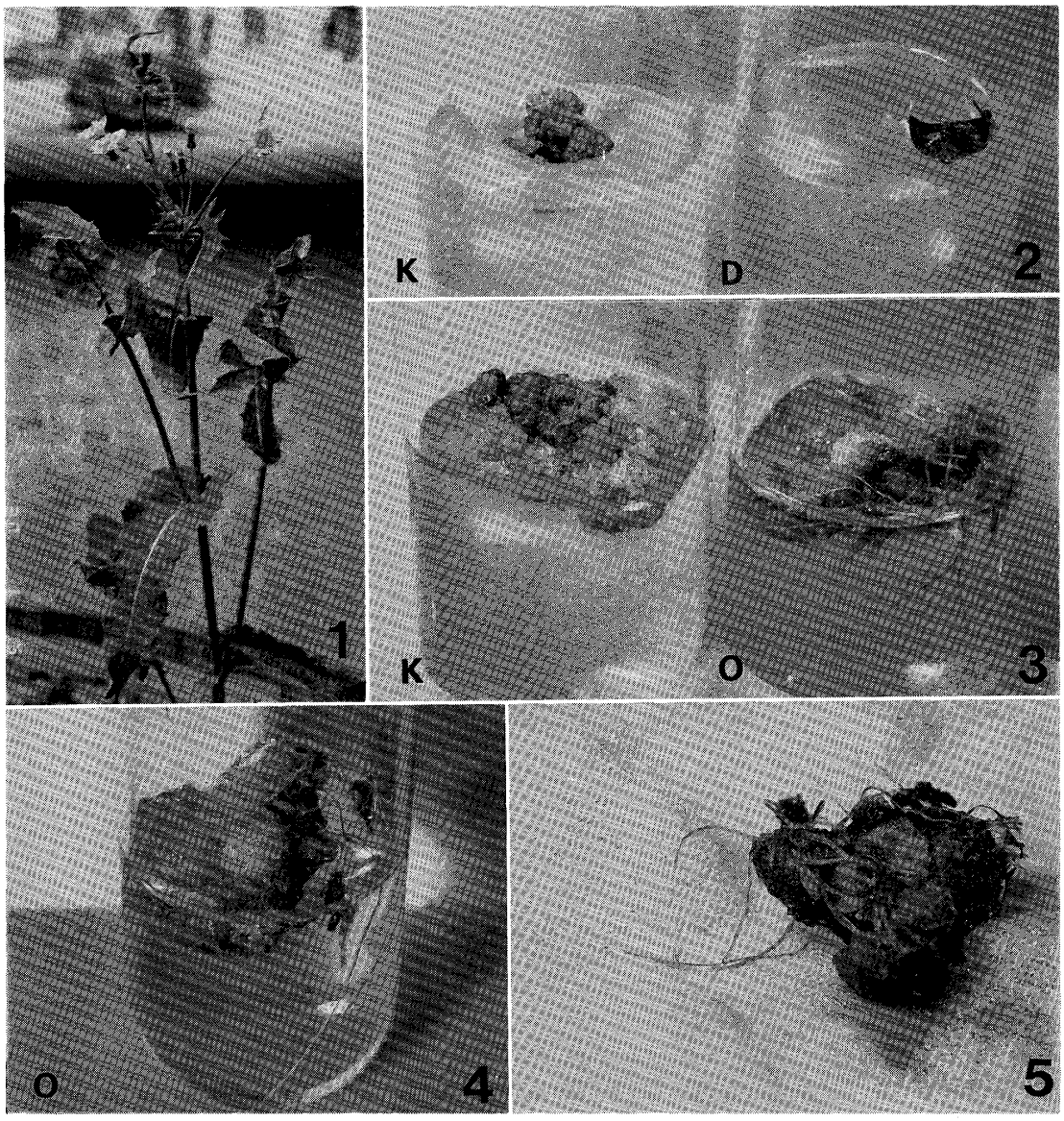
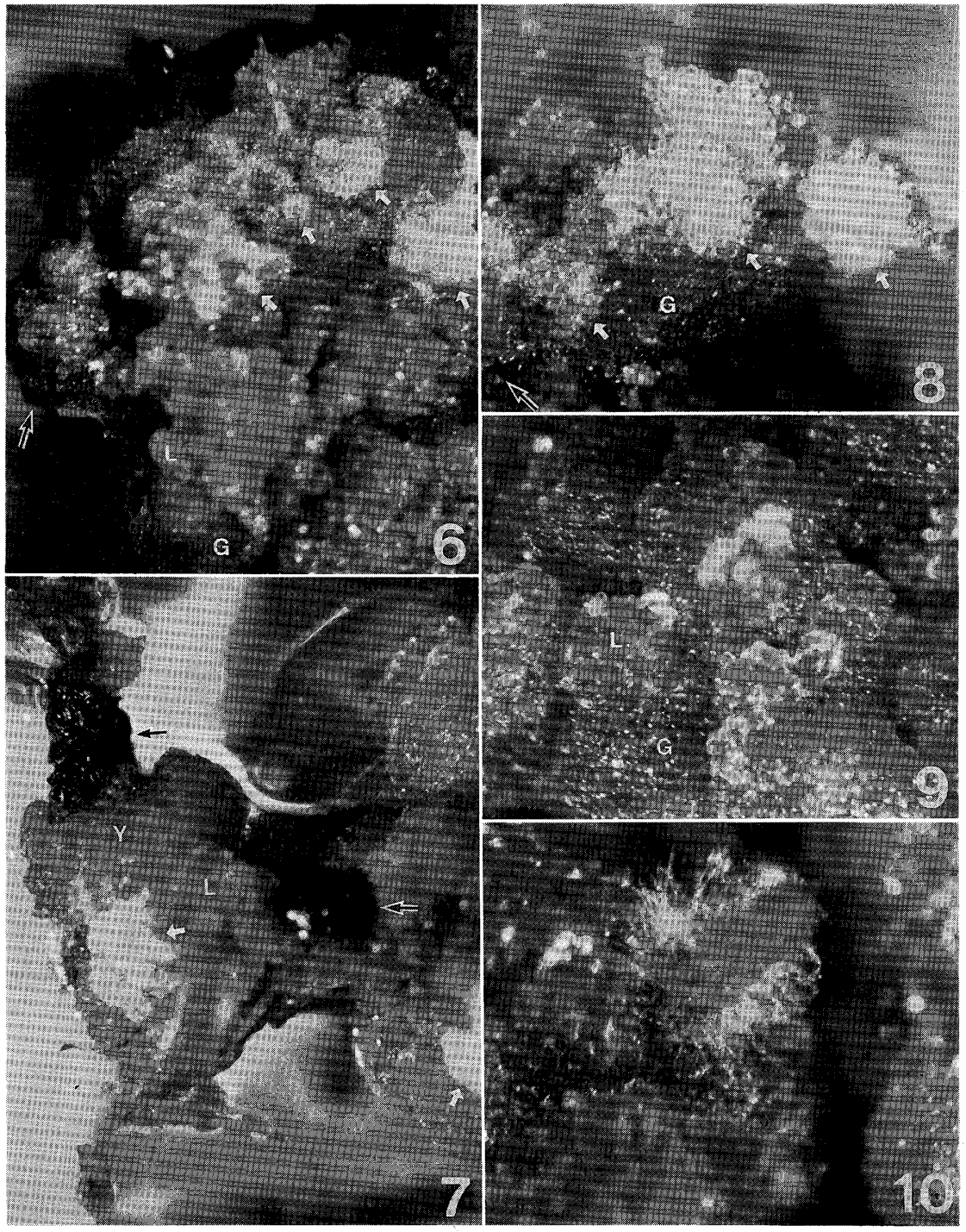


Fig. 1. *Sonchus oleraceus* in experimental field.

Figs. 2–5. Callus induction and differentiation from leaf-explant of *Sonchus oleraceus*.



Figs. 6–10. Differentiation of calli of *Sonchus oleraceus*. ⇨ indicates white callus. ➡ indicates red-brown one. The letter Y shows yellow callus, G the green callus, L light green callus.

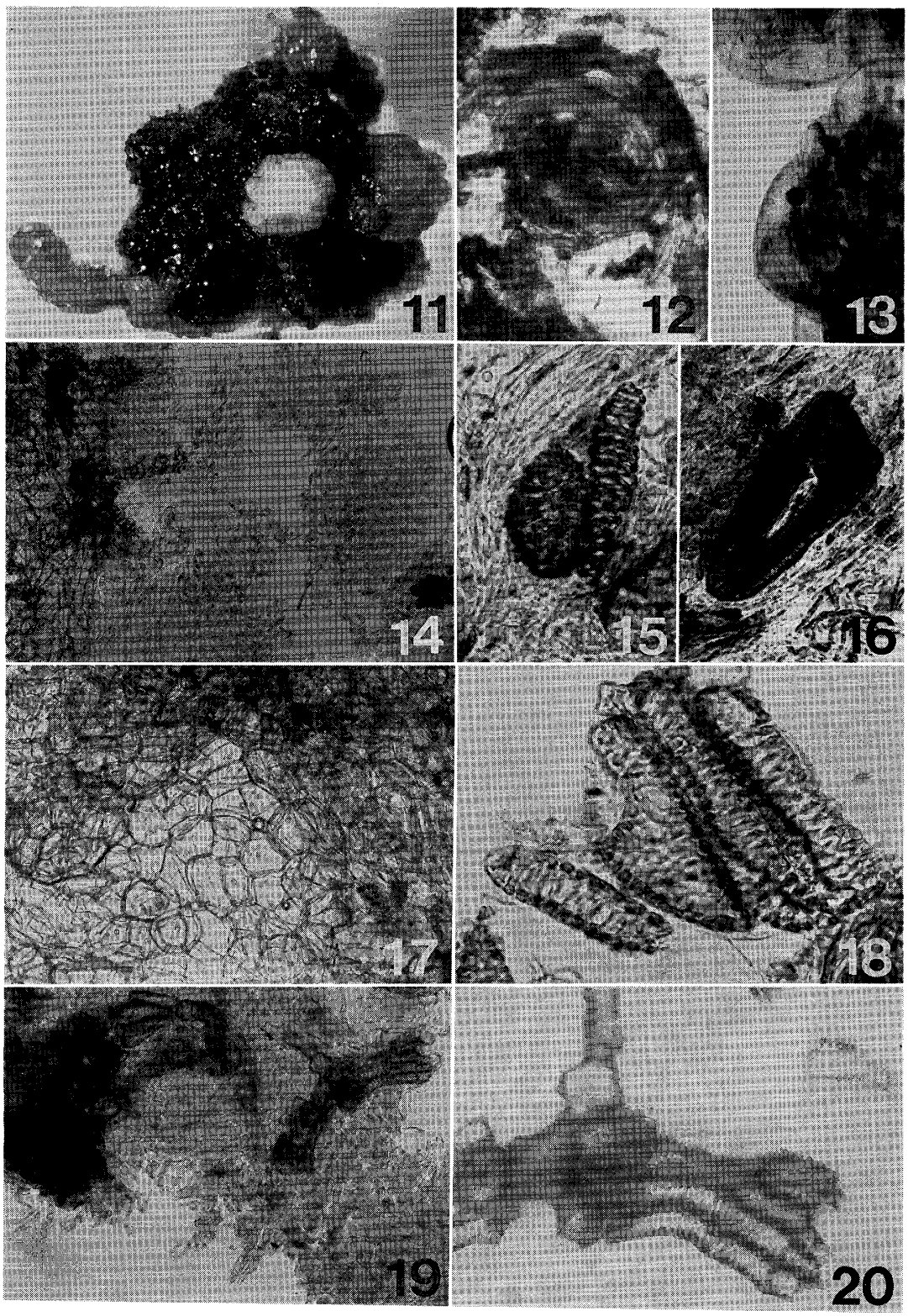


Fig. 11. Callus formation from the leaflet.

Figs. 12–20. Microphotographs of white callus shown in center of Fig. 11.

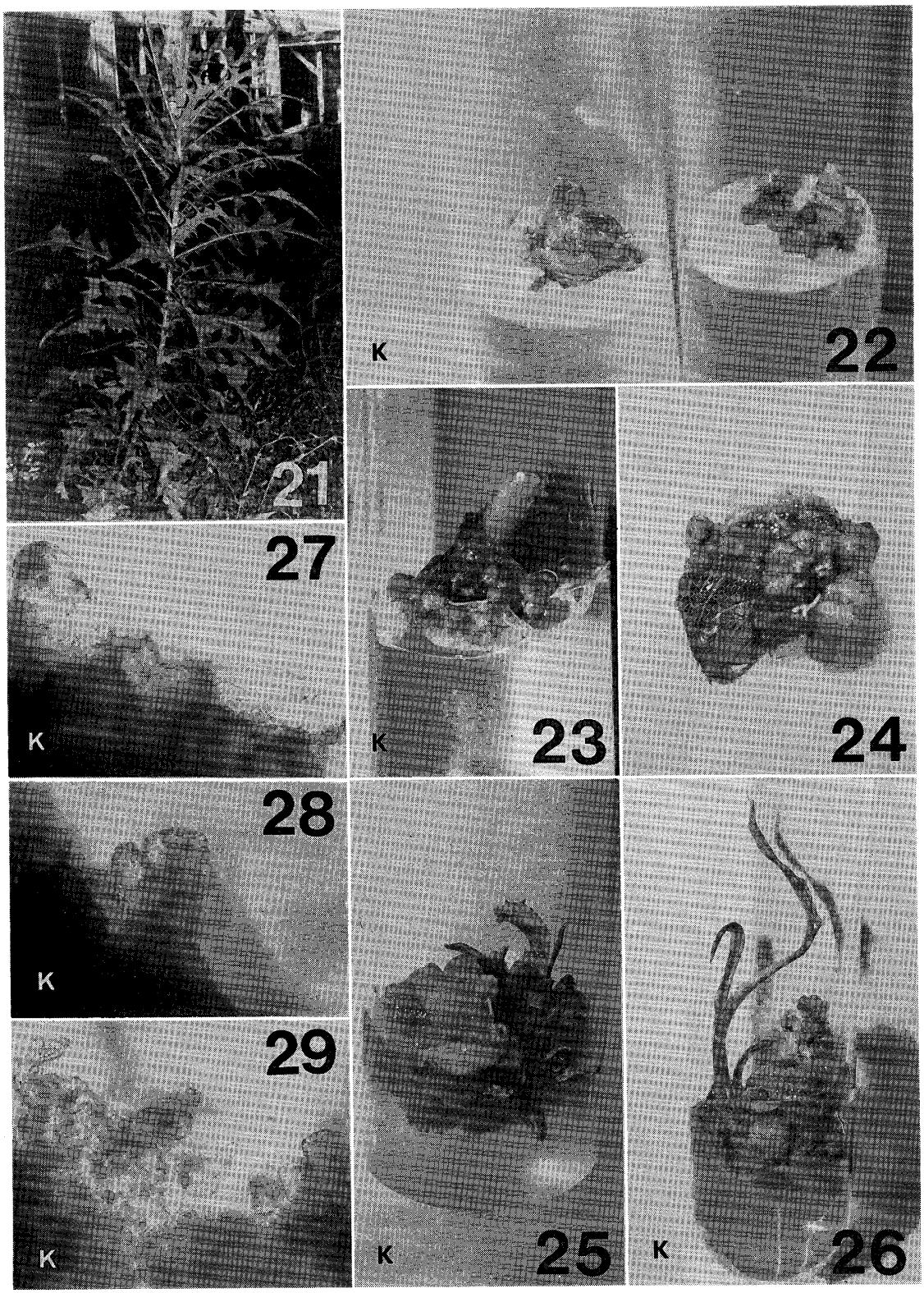
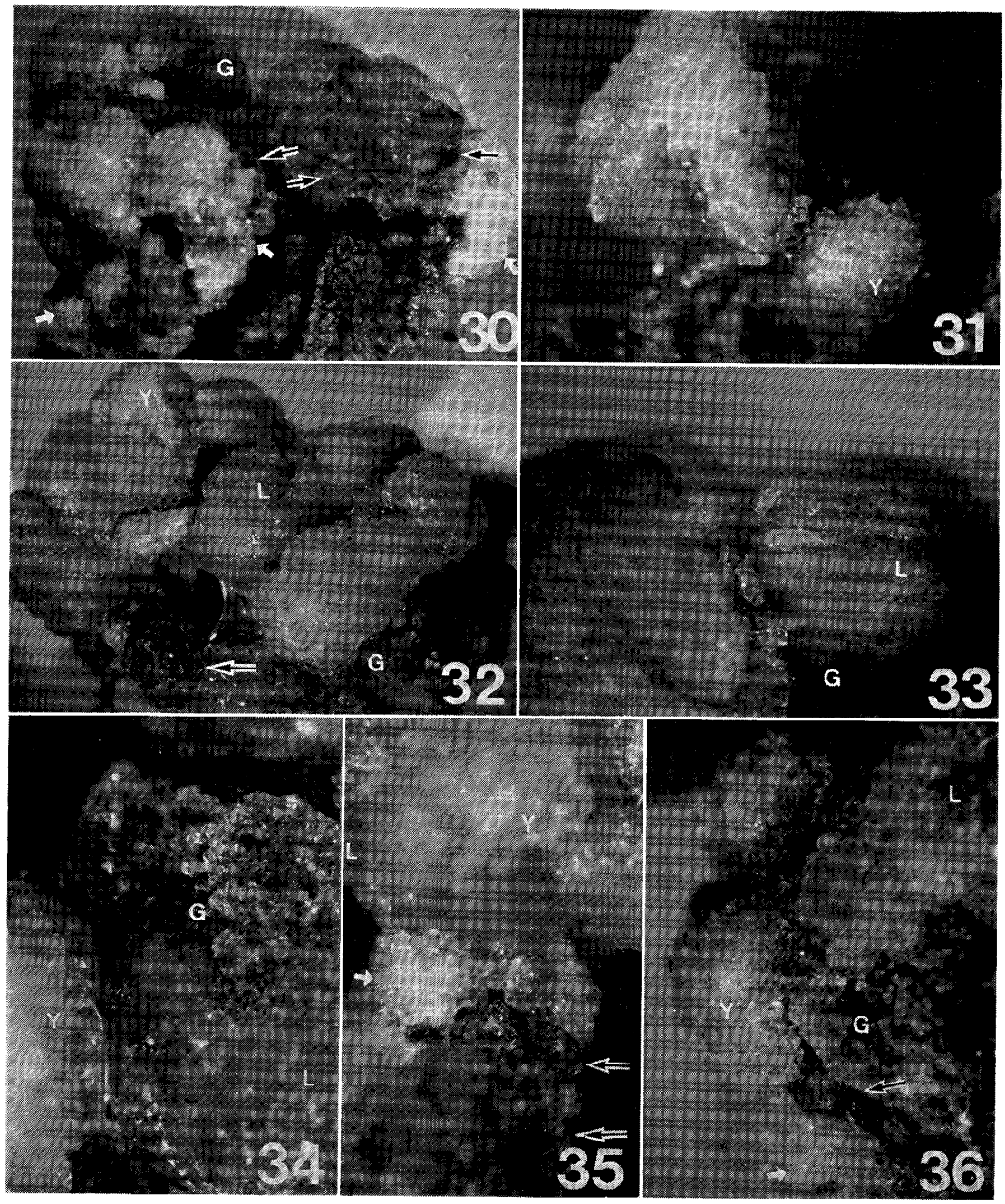
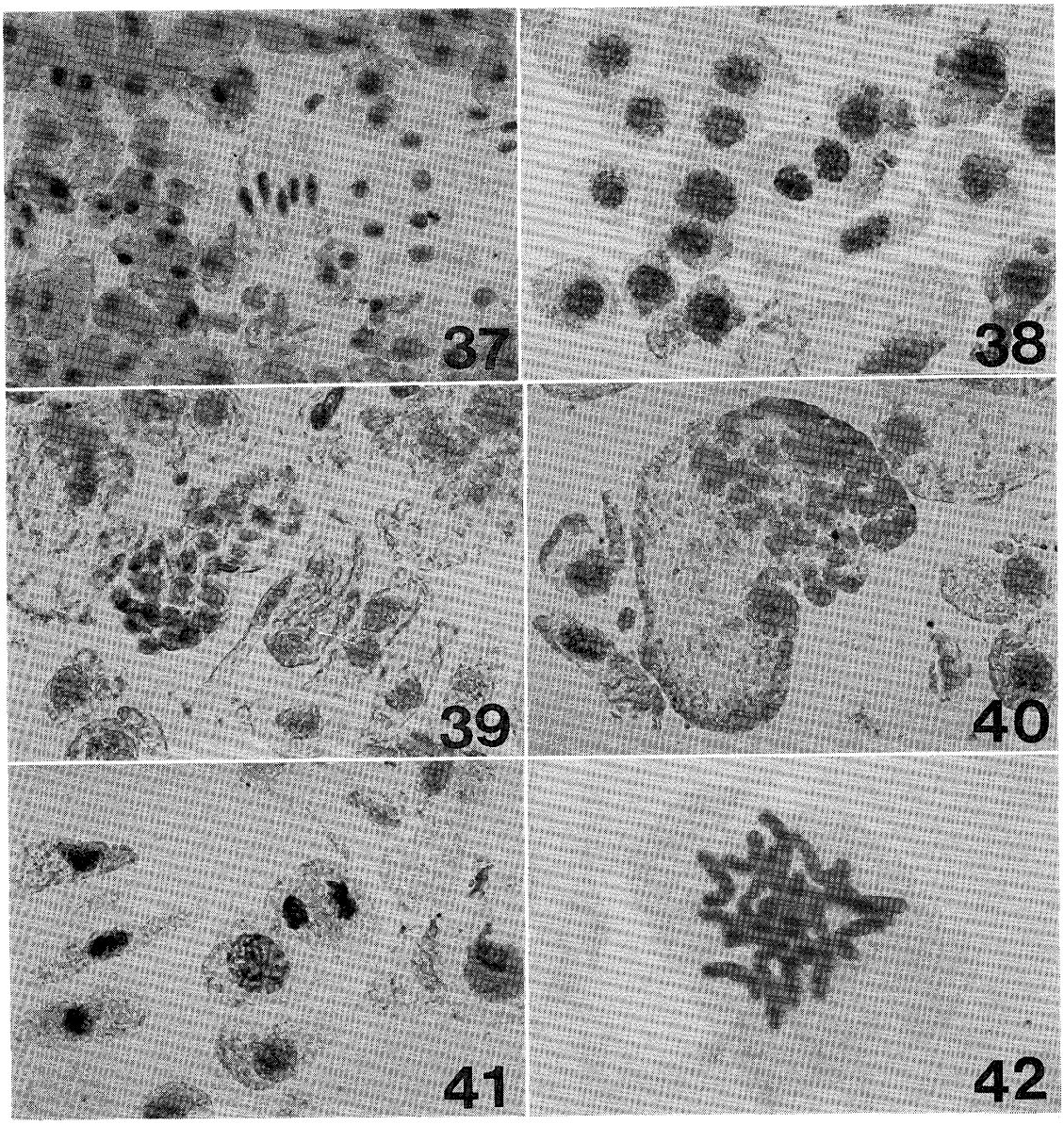


Fig. 21. *Lactuca indica* in experimental field.

Figs. 22–29. Callus induction and leaf formation from leaf explant of *Lactuca indica* on medium K.



Figs. 30–36. Various calli of *Lactuca indica*.



Figs. 37–42. Mitotic cells in calli of *Lactuca indica*.